Investigation of anti-*Neospora caninum* antibodies and disease-related risk factors in goats¹⁾

ARMAGAN ERDEM UTUK, FUNDA ESKI*

Department of Parasitology, *Department of Obstetrics and Gynecology,
Faculty of Ceyhan Veterinary Medicine, University of Cukurova, 01330 Adana, Turkey

Received 19.02.2019

Accepted 06.05.2019

Utuk A. E., Eski F.

Investigation of anti-*Neospora caninum* antibodies and disease-related risk factors in goats

Summary

Neospora caninum is a parasitic protozoan that causes abortion, stillbirth, and premature culling in goats. The aims of this study were (i) to determine the prevalence of anti-*N. caninum* antibodies in goats in the Adana province of Turkey(ii), to identify risk factors for the disease, and (iii) to provide collective data on goat neosporosis. For this purpose, 383 sera were collected from goats of different breeds, ages, and sexes from 15 counties of Adana. A commercially available c-ELISA test kit was used to detect anti-*N. caninum* antibodies. To identify risk factors that influence the prevalence of neosporosis, an oral survey was conducted, and the data collected were evaluated by the logistic regression analysis. The prevalence was determined as 8.9% (34/383) at the individual level and 66.6% (10/15) at the flock level. Statistical analysis indicated that the co-presence of sheep, the animals' being of pure breed and the purchasing of animals from different flocks are the factors that increase the prevalence of neosporosis, while feeder disinfection decreases it.

Keywords: goat, neosporosis, risk factors, c-ELISA

Goat breeding is an important livestock industry in areas without suitable pastures, in macquis groves, and in mountainous regions of Turkey. One of essential components of goat breeding is to rear healthy offspring every year and to maintain the sustainability of the flock. Abortions that cannot be avoided are the biggest problem of goat breeding (30, 31). In goats, abortion is the loss of foetus at any time of gestation, and it usually occurs in the last 2 months of pregnancy. Various stress factors, nutritional disorders, poisonings, hormonal disorders, genetic factors, and infectious agents are major causes of abortion. Infectious agents are bacteria, viruses, fungi, and protozoa (31). According to the classical literature, the most important abortifacient protozoon in small ruminants is *Toxoplasma gondii*. Recent research, however, indicates that *N. caninum* may also be significant (30, 39).

Neospora caninum is a tissue-dwelling parasitic protozoon in the phylum Apicomplexa. Canidae are both the final and intermediate hosts of the parasite, while

ruminants are intermediate hosts. In the domestic cycle of *N. caninum*, the most important final and intermediate hosts are dogs and cows, respectively. Disease can be transmitted both horizontally and vertically. In the horizontal transmission, dogs are infected by eating the bradyzoite-contaminated meat of intermediate hosts, whereas the intermediate hosts are infected by oral uptake of water or food contaminated with sporulated oocysts. In pregnancy, vertical transmission occurs in two ways: as endogenous or exogenous. Endogenous transplacental transmission results from the reactivation of an existing persistent infection within a cow and leads to the birth of a persistently infected calf. Exogenous transplacental transmission, on the other hand, is the infection of a cow with oocysts, which leads to abortion (17, 39, 44). Epidemic, endemic and sporadic abortions occur in infected cattle (32). Furthermore, early foetal deaths, stillbirths, and neonatal mortalities occur due to foetopahtic effects of the parasite. Foetal deaths result in economic losses due to increased calving intervals and delayed lactation. In addition, increased culling of valuable stock and decreased value of herds with high prevalence rates are considered as important problems for animal breeding (38).

¹⁾ This study was supported by the Scientific Research Coordination Unit of Cukurova University as project TSA-2017-8036, and a part of this study was presented orally at International Mediterranean Science and Engineering Congress (IMSEC 2017).

Fig. 1. Location of Adana, study areas and sample number (n)

Recent studies have shown that *N. caninum* causes abortions, foetal deaths, and stillbirths in goats, just as it does in cattle. Histopathologic and molecular techniques are used in the diagnosis of neosporosis, while the prevalence of the diseases is determined by serologic methods (c-ELISA, i-ELISA, NAT, IFAT) (39).

The aims of this study were (i) to determine the prevalence of anti-*N. caninum* antibodies in goats at individual and flock levels in the Adana province of Turkey, (ii) to identify risk factors for the disease, and (iii) to provide collective data on goat neosporosis.

Material and methods

Sera samples were obtained from 383 goats of different breeds, ages, and sexes from 15 counties of Adana, and stored at -20°C until used (Fig. 1). While the counties were grouped according to their altitudes ($< 100\text{ m}$ $> 100\text{ m}$),

the goats were grouped according to breeds (hair goat and others), ages (≤ 3 and ≥ 4 years), and sexes (male and female).

To determine risk factors for neosporosis, the goat owners were asked questions about rearing systems, mix-breeding, pasture type, dominant breed, abortion and reproductive problems, disinfection, animal purchasing, and the presence of dogs. The data collected were evaluated by logistic regression analysis. The results of the statistical analysis are shown as an estimated relative risk (odds ratio-OR) and a 95% confidence interval (CI). The statistical significance level was determined as $P < 0.05$ (Tab. 1).

A commercial c-ELISA test kit (VMRD, USA) was used to detect anti-*N. caninum* antibodies. Samples with percent inhibition values ≥ 30 were confirmed as positive, and those with percent inhibition values < 30 as negative.

This study was approved by the Ethics Committee of Adana Veterinary Control Institute (05.05.2016/1369).

Results and discussion

At the end of the study, the prevalence was determined as 8.9% (34/383) at the individual level and 66.6% (10/15) at the flock level. Percent inhibition values ranged from 30.34056 to 95.41463 in positive samples and from -48.8132 to 29.72136 in negative samples. Statistical analysis showed that the co-presence of sheep, the animals' being of pure breed and the purchasing of animals from different flocks are factors that increase the prevalence of neosporosis, while feeder disinfection is a factor that decreases it ($P < 0.05$) (Tab. 1).

According to the classical literature, *T. gondii* is the most important abortifacient protozoon in small ruminants (30, 31). In the 1990s, *N. caninum* was detected in stillborn and aborted goat fetuses, which created the awareness of goat neosporosis. After the 2000s, stud-

ies focused on risk factors, prevention, and prevalence rates of *N. caninum* in goat flocks from different countries (39). Although there have been many studies on cattle neosporosis in recent decades, neosporosis has not been sufficiently investigated in other livestock and wild animals. Economic losses caused by *N. caninum* and the epidemiological characteristics of the parasite still remain unknown (3, 17, 32).

According to different serological studies, the prevalence of the disease amounted to 6% in Africa, 0.7-7.23% in Asia, 0.47-15.5% in Europe, 3.8-5.8% in North America, 1.05-17.7% in South America (Tab. 2) and 0-25.9% in Turkey (Tab. 3). According to our global assessment, the average seroprevalence of goat neosporosis was 4.54% (118/2598) in Asia, 3.88% (147/3781) in Europe, 4.68% (15/320) in North America, 7.29% (684/9374) in South America, 5.99%

Tab. 1. Risk factors for *Neospora caninum* infection in goats according to logistic regression analysis

Variable	Category	No. tested	No. positive	% positive	Odds ratio	95% CL	X ²	P Value
Altitude	< 100 m	179	17	9.5	1.14	0.60-2.16	0.160	0.689
	> 100 m	204	17	8.3				
Breed	Hair goat	230	17	7.4	1.56	0.77-3.17	1.572	0.210
	Others	153	17	11.4				
Sex	Female	341	31	9.1	1.27	0.40-3.98	0.175	0.675
	Male	42	3	7.1				
Age	≤ 3 years	192	16	8.3	1.14	0.56-2.31	0.141	0.707
	≥ 4 years	191	18	9.4				
Rearing system	Semi-extensive	358	31	8.7	1.43	0.41-5.07	0.322	0.570
	Intensive	25	3	12.0				
Co-presence of sheep	Yes	173	22	12.7	2.22	1.13-4.36	5.750	0.016*
	No	210	12	5.7				
Pasture type	Common	329	32	9.4	1.70	0.54-5.35	0.857	0.354
	Own	74	2	5.6				
Dominant breed	Pure	309	32	10.4	3.83	0.93-15.63	4.323	0.038*
	Crossbreed	74	2	2.7				
History of abortion	Yes	256	26	10.2	1.61	0.75-3.45	1.561	0.211
	No	127	8	6.3				
History of postpartum problems	Yes	172	16	9.3	1.09	0.57-2.07	0.070	0.792
	No	211	18	8.5				
History of infertility	Yes	169	19	11.2	1.60	0.84-3.06	2.092	0.148
	No	214	15	7.0				
Shelter disinfection	No	148	18	12.2	1.79	0.93-3.85	3.209	0.070
	Yes	235	36	6.8				
Feeder disinfection	No	334	34	10.2	1.11	1.07-1.15	5.474	0.019*
	Yes	49	0	0				
Animal purchasing	No	304	22	7.2	2.29	1.08-4.87	4.903	0.027*
	Yes	79	12	15.2				
Dogs around the feeders	Yes	292	26	8.9	1.01	0.47-2.15	0.001	0.970
	No	91	8	8.8				

Explanation: * – $P < 0.05$

Tab. 2. Serological studies on caprine neosporosis in different parts of the world

Continent	Country	Region	Test	Kit	Cut-off	SE	SP	No. tested	No. positive	% positive	Reference
Africa	Sudan	Khartoum state	c-ELISA	VMRD	≥ 30	–	–	100	6	6	(20)
	China	Qinghai province	i-ELISA, IFAT*	IDEXX	?	98.6	98.3	650	47	7.23	(24)
	Iraq	Wasit province	i-ELISA	IDvet	≥ 50	–	–	106	6	5.6	(16)
Asia	Iranian	Hamedan province	i-ELISA	IDvet	≥ 50	–	–	450	28	6.2	(15)
	Jordan	Northern Jordan	i-ELISA	BIO-X	–	95	96	302	–	2 (CTP)	(1)
	Jordan	Southern Jordan	i-ELISA	IDEXX Chekit	?	97.5	95.1	300	17	5.7 (CTP)	(2)
	Korea	Northern, central and southern regions	i-ELISA	IDEXX	?	97.6	98.5	464	4	0.9	(22)
	Pakistan	Punjab	c-ELISA	VMRD	≥ 30	96	99	142	13	8.6	(29)
Europe	Sri Lanka	Various parts of the country	ih-ELISA, IFAT*, WB*	–	?	–	–	486	3	0.7	(28)
	Germany	Hesse	i-ELISA	IDvet	≥ 50	–	–	415	2	0.48	(42)
	Czech Republic	Eight different regions	c-ELISA, IFAT*	VMRD	≥ 30	–	–	251	15	6	(7)
	Greece	Various regions	ih-ELISA	–	?	–	–	375	26	6.9	(4)
	Italy	Milan, Bergamo, Varese	ih-ELISA, WB*	–	?	–	–	414	24	5.7	(14)
	Poland	Entire country	i-ELISA, IFAT*	IDEXX Chekit	?	98.6	98.3	1060	5	0.47	(10)
	Romania	Four different regions	i-ELISA	IDEXX Chekit	≥ 50	98.6	98.3	512	12	2.3	(21)
	Slovakia	Eastern Slovakia	c-ELISA	VMRD	≥ 30	–	–	116	18	15.5	(9)
	Spain	Galicia	c-ELISA	VMRD	≥ 30	–	–	638	45	6	(11)
	Eastern Caribbean	Grenada	i-ELISA	IDvet	?	100	100	138	8	5.8	(35)
North America	Mexico	Veracruz	iELISA	IDEXX	?	100	98.9	182	7	3.8	(19)
South America	Argentina	Córdoba, Buenos Aires	IFAT	–	1 : 50	–	–	1594	106	6.6	(26)
	Argentina	La Rioja Province	IFAT	–	1 : 100	–	–	2922	162	5.5	(18)
	Brazil	Paraná State	IFAT	–	1 : 50	–	–	306	10	3.3	(12)
	Brazil	Bahia State	IFAT	–	1 : 100	–	–	384	58	15	(41)
	Brazil	Minas Gerais State	IFAT	–	1 : 50	98	99	667	–	10.7 (CTP)	(5)
	Brazil	Maranhão State	IFAT	–	1 : 25	–	–	46	8	17.39	(27)
	Brazil	Paraná State	IFAT	–	1 : 50	–	–	975	26	2.7	(33)
	Brazil	Santa Catarina State	IFAT	–	1 : 50	–	–	654	30	4.58	(37)
	Brazil	São Paulo State	NAT	–	1 : 25	–	–	923	161	17.7	(25)
	Brazil	Pernambuco State	IFAT	–	1 : 50	–	–	174	5	2.9	(6)
	Brazil	Paraná State	IFAT	–	1 : 50	–	–	202	4	2	(6)
	Brazil	São Paulo State	IFAT	–	1 : 50	–	–	394	25	6.4	(13)
	Brazil	Pernambuco State	IFAT	–	1 : 50	–	–	319	85	26.6	(36)
	Brazil	Rio Grande do Norte State	IFAT	–	1 : 50	–	–	381	4	1.05	(23)

Explanation: * – confirmation tests; c-ELISA – Competitive Enzyme-Linked Immunosorbent Assay; i-ELISA – Indirect ELISA; ih-ELISA – In house ELISA; WB – Western Blotting; IFAT – Indirect Fluorescent Antibody Test; NAT – Neospora Agglutination Test; SE – Sensitivity; SP – Specificity; CTP – Corrected true seroprevalence

Tab. 3. Serological studies on caprine neosporosis in Turkey

Region	Province	Test	Kit	Cut-off	SE	SP	No. tested	No. positive	% positive	Reference	Mean Prevalence (%)	Turkey (%)
Central Anatolia	Kırşehir	c-ELISA	VMRD	≥ 30	96	99	41	1	2.43	(40)	13.62 (56/411)	14.44 (91/630)
	Konya	ih-ELISA	–	–	–	–	189	8	4.2	(45)		
	Niğde	c-ELISA	VMRD	≥ 30	–	–	181	47	25.9	(8)		
Eastern Anatolia	Elazığ	c-ELISA	VMRD	≥ 30	96	99	70	8	11.4	(40)	13.79 (12/87)	
	Erzurum	c-ELISA	VMRD	≥ 30	96	99	17	4	23.5	(40)		
Mediterranean	Karaman	ih-ELISA	–	–	–	–	60	0	0	(45)	0 (0/60)	
Southeastern Anatolia	Kilis	c-ELISA	VMRD	≥ 30	96	99	92	14	15.21	(39)	8.45 (23/72)	
	Şanlıurfa	c-ELISA	VMRD	≥ 30	–	–	180	9	5	(34)		

Explanations: c-ELISA – Competitive Enzyme-Linked Immunosorbent Assay; ih-ELISA – In house ELISA; SE – Sensitivity; SP – Specificity

Tab. 4. Seroprevalence of goat neosporosis on particular continents and in the world

Continent	No. tested	No. positive	% positive
Africa	100	6	6.00
Asia	2598	118	4.54
Europe	3781	147	3.88
North America	320	15	4.68
South America	9374	684	7.29
Total (World)	16173	970	5.99

(970/16173) globally (Tab. 4), and 14.44% (91/630) in Turkey (Tab. 3). In this study, the individual prevalence was determined as 8.9% (34/383), which is below the average for Turkey (14.44%), but above the world's average (5.99%).

Flock-level prevalence was reported as 12-50% in Jordan (1, 2), 13.3% in Iran (15), 32.1% in Italy (14), 0.9% in Poland (9), 38% in Spain (11), 53.2% in Argentina (26) and 16.4-75.2% in Brazil (5, 33). In this study, we determined the flock-level prevalence as 66.6% (10/15), which is higher than the above-mentioned rates, except for Brazil (5). Both individual and flock-level prevalence determined in the present study are above the world's average, and our results suggest that goat neosporosis is endemic in Adana and Turkey.

In previous studies, the relationship between the disease and various risk factors was examined, and conflicting results were obtained. In some studies, the age, breed, presence of dogs in flocks, and season are presented as risk factors important for the prevalence of neosporosis, which is not confirmed by some other studies (1, 2, 6, 8, 13, 14, 16, 22, 26, 34, 37, 39, 41). As a general consensus, the sex of the animals is thought to have no effect on the prevalence of the disease (6, 12, 14-16). In this study, statistical analysis indicates that the co-presence of sheep, the animals' being of pure breed and the purchasing of animals from different flocks are factors that increase the prevalence of neosporosis, while feeder disinfection decreases it ($P < 0.05$) (Tab. 1).

Dramatic differences in the results of serological studies are due to different serological tests, cut-off values, specificity, and sensitivity rates, as well as wrong sampling (39, 40). Low cut-off values result in high sensitivity and low specificity, while high values have opposite effects (43). With regard to the ELISA kits and IFA tests used in different serological studies, it can be observed that cut-off values range from 30 to 50, dilutions from 1/25 to 100, sensitivities of ELISAs from 95% to 100%, and specificities from 95.1% to 100% (Tab. 2). It should be kept in mind that non-optimized serological tests may result in false positivity or negativity, and wrong sampling may also have adverse effects on test results.

In conclusion, to understand the epidemiology of neosporosis and to develop appropriate protection and control strategies, it is important to ensure harmonization among laboratories, to use the same test methods, cut-off values, and dilution ratios, as well as tests with the same specificity and sensitivity rates, and to keep proper records of the determinants of the disease related to the species, host, and environment in large scale studies at national and regional levels.

References

1. Abo-Shehadeh M. N., Abu-Halaweh M. M.: Flock-level seroprevalence of, and risk factors for, *Neospora caninum* among sheep and goats in northern Jordan. *Prev. Vet. Med.* 2010, 93, 25-32.
2. Al-Majali A. M., Jawasreh K. I., Talafha H. A., Talafha A. Q.: Neosporosis in sheep and different breeds of goats from southern Jordan: prevalence and risk factors analysis. *Am. J. Anim. Vet. Sci.* 2008, 3, 47-52.
3. Almeria S.: *Neospora caninum* and wildlife. *ISRN Parasitology* 2013, 1-23, <http://dx.doi.org/10.5402/2013/947347>.
4. Anastasia D., Elias P., Nikolaos P., Charilaos K., Nektarios G.: *Toxoplasma gondii* and *Neospora caninum* seroprevalence in dairy sheep and goats mixed stock farming. *Vet. Parasitol.* 2013, 198, 387-390.
5. Andrade G. S., Bruhn F. R. P., Rocha C. M. B. M., Sa Guimaraes A., Gouveia A. M., Guimaraes A. M.: Seroprevalence for *Neospora caninum* in goats of Minas Gerais State, Brazil. *Res. Vet. Sci.* 2013, 94, 584-586.
6. Arraes-Santos A. L., Araújo A. C., Guimarães M. F., Santos M. F., Pena J. R., Gennari H. F. J., Azevedo S. M., Labruna S. S., Horta M. B., Maurício C.: Seroprevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in domestic mammals from two distinct regions in the semi-arid region of Northeastern Brazil. *Vet. Parasitol. (Amst.)* 2016, 5, 14-18.
7. Bartova E., Sedlak K.: *Toxoplasma gondii* and *Neospora caninum* antibodies in goats in the Czech Republic. *Vet. Med.* 2012, 57, 111-114.
8. Cayvaz M., Karatepe M.: Niğde yöresi keçilerinde *Neospora caninum*'un seroprevalansı. *Kafkas Univ. Vet. Fak. Derg.* 2011, 17, 935-939.

9. Czopowicz M., Kaba J., Szaluś-Jordanow O., Nowicki M., Witkowski L., Frymura T.: Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infections in goats in Poland. *Vet. Parasitol.* 2011, 178, 339-341.
10. Čobádiová A., Reiterová K., Derdákova M., Špilovská, S., Turčeková L., Hviščová I., Hisira V.: *Toxoplasma gondii*, *Neospora caninum* and tick-transmitted bacterium *Anaplasma phagocytophilum* infections in one selected goat farm in Slovakia. *Acta Parasitol.* 2013, 58, 541-546.
11. Díaz P., Cabanelas E., Díaz J. M., Viña M., Béjar J. P., Pérez-Creo A., Prieto A., López C. M., Panadero R., Fernández G.: Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in goats from North-Western Spain. *Ann. Agric. Environ. Med.* 2016, 23, 587-590.
12. Faria E. B., Gennari S. M., Pena H. F., Athayde A. C. R., Silva M. L. C. R., Azevedo S. S.: Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in goats slaughtered in the public slaughterhouse of Patos City, Paraíba State, Northeast region of Brazil. *Vet. Parasitol.* 2007, 149, 126-129.
13. Figliuolo L. P. C., Rodrigues A. A. R., Viana R. B., Rodrigues A. A. R., Viana R. B., Aguiar D. M., Kasai N., Gennari S. M.: Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in goat from São Paulo State, Brazil. *Small Ruminant. Res.* 2004, 55, 29-32.
14. Gazzonis A. L., Garcia G. A., Zanzani S. A., Mora L. M. O., Invernizzi A., Manfredi M. T.: *Neospora caninum* infection in sheep and goats from North-Eastern Italy and associated risk factors. *Small Ruminant. Res.* 2016, 140, 7-12.
15. Gharekhani J., Esmailnejad B., Rezaei H., Yakhchali M., Heidari H., Azhari M.: Prevalence of anti-*Neospora caninum* antibodies in Iranian goats. *Ann. Parasitol.* 2016, 62, 111-114.
16. Ghattaf H. H., Faraj A. A.: Seroprevalence of *Neospora caninum* in goats in Wasit Province, Iraq. *I.J.C.M.A.S.* 2015, 4, 182-191.
17. Goodswen S. J., Kennedy P. J., Ellis J. T.: A review of the infection, genetics, and evolution of *Neospora caninum*: from the past to the present. *Infect. Genet. Evol.* 2013, 13, 133-150.
18. Gos M. L., Manazza J. A., Späth E. J. A., Pardini L., Fiorentino M. A., Unzaga J. M., Moré G., Venturini M. C.: Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infections in goats from two Argentinean provinces. *Open Vet. J.* 2017, 7, 319-322.
19. Huerta-Peña J. C., Martínez-Herrera D. I., Peniche-Cardena Á. E. J., Villanueva-Valencia M., Hernández-Ruiz S. G., Villagómez-Cortés J. A., Barradas-Piña F. T., Morales-Álvarez J. F., Flores-Castro R.: Seroprevalence and risk factors associated with *Neospora caninum* in goats from municipalities of the central region of Veracruz. *Trop. Subtrop. Agroecosyst.* 2011, 13, 445-454.
20. Ibrahim A. M., Ismail A. A., Angara T. E. E.: Analysis of risk factors associated with seroprevalence of *Toxoplasma gondii* in dairy animals from Khartoum State, Sudan. *Sudan J. Sc. Tech.* 2015, 16, 19-28.
21. Iovu A., Györke A., Mircean V., Gavrea R., Cozma V.: Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dairy goats from Romania. *Vet. Parasitol.* 2012, 186, 470-474.
22. Jung B., Lee S., Kwak D.: Evidence of *Neospora caninum* exposure among native Korean goats (*capra hircus coreanae*). *Vet. Med.* 2014, 59, 637-640.
23. Lima J. T. R., Ahid S. M. M., Júnior R. A. B., Pena H. F. J., Dias R. A., Gennari S. M.: Prevalência de anticorpos anti-*Toxoplasma gondii* E anti-*Neospora caninum* em rebanhos caprinos do município de Mossoró, Rio Grande Do Norte. *Braz. J. Vet. Res. An. Sci.* 2008, 45, 81-86.
24. Liu Z. K., Li J. Y., Pan H.: Seroprevalence and risk factors of *Toxoplasma gondii* and *Neospora caninum* infections in small ruminants in China. *Prev. Vet. Med.* 2015, 118, 488-492.
25. Modolo J. R., Stachissini A. V. M., Gennari S. M., Dubey J. P., Langoni H., Padovani C. R., Barrozo L. V., Leite B. L. S.: Frequência de anticorpos anti-*Neospora caninum* em soros de caprinos do estado de São Paulo e sua relação com o manejo dos animais. *Pesqui. Vet. Bras.* 2008, 28, 597-600.
26. Moore D. P., Yaniz M. G., Odeón A. C., Cano D., Leunda M. R., Späth E. A. J., Campero C. M.: Serological Evidence of *Neospora caninum* Infections in goats from La Rioja Province, Argentina. *Small Rum. Res.* 2007, 73, 256-258.
27. Moraes L. M. B., Raimundo J. M., Guimarães A., Santos H. A., Junior M., de Lima G., Massard C. L., Machado R. Z., Baldani C. D.: Occurrence of anti-*Neospora caninum* and anti-*Toxoplasma gondii* IgG Antibodies in goats and sheep in Western Maranhão, Brazil. *Rev. Bras. Parasitol.* V. 2011, 20, 312-317.
28. Naguleswaran A., Hemphill A., Rajapakse R. P., Sager H.: Elaboration of a crude antigen ELISA for serodiagnosis of caprine neosporosis: Validation of the test by detection of *Neospora caninum*-specific antibodies in goats from Sri Lanka. *Vet. Parasitol.* 2004, 126, 257-262.
29. Nasir A., Ashraf M., Khan M. S., Javed A., Yaqub T., Avais M., Reichel M. P.: Prevalence of *Neospora caninum* antibodies in sheep and goats in Pakistan. *J. Parasitol.* 2012, 98, 213-215.
30. Noakes D. E., Parkinson T. J., England G. C. W.: Infertility in the ewe and doe (female goat), [in:] *Arthur's Veterinary Reproduction and Obstetrics*. 8th Ed, Bailliere Tindall, London 2001, p. 570-575.
31. Pugh D. G.: *Therigenology of sheep and goats*, [in:] *Sheep and Goat Medicine* 1st Ed. Saunders, Philadelphia 2002, p. 175.
32. Reichel M. P., Ayanegui-Alcerrec M. A., Gondim L. F., Ellis J. T.: What is the global economic impact of *Neospora caninum* in cattle – the billion-dollar question? *Int. J. Parasitol.* 2013, 43, 133-142.
33. Santos C. S. A. B., Azevedo S. S., Soares H. S., Higino S. S. S., Santos F. A., Silva M. L. C. R., Pena H. F. J., Alves C. J., Gennari S. M.: Flock-level risk factors associated with *Neospora caninum* seroprevalence in dairy goats in a semiarid region of Northeastern Brazil. *Small Rum. Res.* 2013, 112, 239-242.
34. Sevgili M., Çimta İ., Keski O.: Şanlıurfa yöresindeki keçilerde *Neospora caninum* enfeksiyonunun seroprevalansı. *Türkiye Parazit. Derg.* 2003, 27, 249-251.
35. Sharma R. N., Bush J., Tiwari K., Chikweto A., Bhaiyat M. I.: Seroprevalence of *Neospora caninum* in sheep and goats from Grenada, West Indies. *Open J. Vet. Med.* 2015, 5, 219-223.
36. Tembue A. A. S. M., Ramos R. A. N., Sousa T. R., Albuquerque A. R., Costa A. J., Meunier I. M. J., Faustino M. A. G., Alves L. C.: Serological survey of *Neospora caninum* in small ruminants from Pernambuco State, Brazil. *Rev. Bras. Parasitol.* V. 2011, 20, 246-248.
37. Topazio J. P., Weber A., Camillo G., Vogel F. F., Machado G., Ribeiro A., Moura A. B., Lopes L. S., Tonin A. A., Soldá N. M.: Seroprevalence and risk factors for *Neospora caninum* in goats in Santa Catarina State, Brazil. *Rev. Bras. Parasitol.* V. 2014, 23, 360-366.
38. Trees A. J., Davison H. C., Innes E. A., Wastling J. M.: Towards evaluating the economic impact of bovine neosporosis. *Int. J. Parasitol.* 1999, 29, 1195-1200.
39. Utuk A. E., Eski F.: Detection of anti-*Neospora caninum* antibodies in a goat flock in Kilis Province of Turkey. *I.J.V.S.* 2017, 6, 114-117.
40. Utuk A. E., Simsek S., Piskin F. C., Balkaya I.: Detection of *Neospora caninum* IgG antibodies in goats in Elazığ, Erzurum and Kırşehir Provinces of Turkey. *Isr. J. Vet. Med.* 2011, 66, 157-159.
41. Uzêda R. S., Pinheiro A. M., Fernández S. Y., Ayres M. C. C., Gondim L. F. P., Almeida M. A. O.: Seroprevalence of *Neospora caninum* in dairy goats from Bahia, Brazil. *Small Rum. Res.* 2007, 70, 257-259.
42. Villagra-Blanco R., Wagner H., Dolz G., Romero-Zúñiga J. J., Taubert A., Wehrend A., Hermosilla C.: First report on the seroprevalence of *Neospora caninum* in goats from the Federal State of Hesse, Germany. *Berl. Munch. Tierarztl. Wschr.* 2017, 130, 517-522.
43. Wapenaar W., Barkema H. W., Vanleeuwen J. A., McClure J. T., O'Handley R. M., Kwok O. C., Thulliez P., Dubey J. P., Jenkins M. C.: Comparison of serological methods for the diagnosis of *Neospora caninum* infection in cattle. *Vet. Parasitol.* 2007, 143, 166-173.
44. Williams D. J. L., Hartley C. S., Bjorkman C., Trees A. J.: Endogenous and exogenous transplacental transmission of *Neospora caninum* – how the route of transmission impacts on epidemiology and control of disease. *Parasitology* 2009, 136, 1895-1900.
45. Zhou M., Cao S., Sevinc F., Sevinc M., Ceylan O., Liu M., Wang G., Moumouni P. F. A., Jirapatharasate C., Suzuki H., Nishikawa Y., Xuan X.: Enzyme-linked immunosorbent assays using recombinant TgSAG2 and NcSAG1 to detect *Toxoplasma gondii* and *Neospora caninum*-specific antibodies in domestic animals in Turkey. *J. Vet. Med. Sci.* 2016, 78, 1877-1881.

Corresponding author: Dr. Funda Eski, Department of Obstetrics and Gynecology, Faculty of Ceyhan Veterinary Medicine, Cukurova University, 01330, Adana, Turkey; e-mail: fndeski@hotmail.com